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EVIDENCE THAT THE T CELL ANTIGEN RECEPTOR
MAY NOT BE INVOLVED IN CYTOTOXICITY MEDIATED BY
 γ/δ AND α/β THYMIC CELL LINES

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The antigen receptor expressed on most thymocytes and mature T lymphocytes is a CD3-associated disulfide-linked heterodimer composed of α and β subunits (1). A minor population of CD3⁺ thymocytes and T cells lack expression of the TCR- α/β (2–4), but instead express a TCR- γ that is often either covalently or noncovalently associated with TCR- δ (2–10). Some IL-2-dependent T cell lines expressing TCR- γ/δ mediate cytotoxicity against a broad panel of tumor cell targets (7–9). The generation of cytotoxicity does not require deliberate immunization and is not restricted by the MHC. Although these studies clearly indicate that T cells expressing TCR- γ/δ possess the cellular machinery to mediate cytotoxicity, it is unclear whether the broad spectrum of cytotoxicity mediated by these cells in vitro reflects their activity in vivo, or alternatively, if this results from in vitro culture. Cytotoxicity mediated by some CD3⁺, γ/δ T cell lines is partially inhibited by anti-CD3 antibodies (7–9). However, antibodies against CD3 may influence the function of a cell by a mechanism other than interfering with antigen recognition. We have established IL-2-dependent cell lines from thymocytes expressing either TCR- α/β or γ/δ and have undertaken studies to determine whether the TCR is necessary for cytotoxic function.

Materials and Methods

Cell Lines. Normal human thymocytes were cultured as described (5). After 2–3 wk, cells were stained with FITC-conjugated WT31 and PE-conjugated anti-Leu-4 (CD3) (5). CD3⁺, WT31⁺ and CD3⁺, WT31[–] thymocytes (hereafter referred to as CD3- α/β and CD3- γ/δ thymocytes, respectively) were isolated to >98% purity using a FACS and were maintained as IL-2-dependent cell lines. KW anti-JY CTL is an IL-2-dependent CD3- α/β , CD8⁺ CTL cell line directed against HLA-A2 (11).

Antibodies. WT31 reacts with a framework determinant on the TCR- α/β (11). W6/32 hybridoma (IgG2a mAb directed against a monomorphic epitope expressed on HLA-A,B,C) was obtained from the American Type Culture Collection (Rockville, MD). F(ab')₂ fragments were prepared by pepsin digestion (12).

Modulation. Modulation was performed as described (13). Briefly, cells were cultured overnight in the presence or absence of anti-Leu-4 mAb. After modulation, cells were washed extensively and were stained by indirect immunofluorescence with IgG1 control

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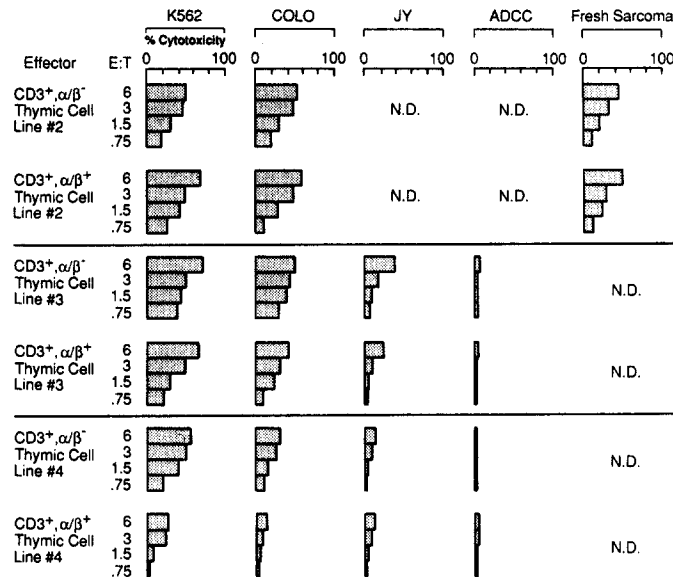


FIGURE 1. Cytotoxicity mediated by CD3- α/β and CD3- γ/δ thymic cell line.

mAb or anti-Leu-4, followed by FITC goat anti-mouse Ig. Cells were analyzed by flow cytometry.

Cytotoxicity Assays. Cytotoxicity was measured using a 4-h radioisotope release assay (14). In antibody-blocking assays, effector cells were incubated at 37°C for 3 h with antibodies before the addition of targets. Antibody-dependent, cell-mediated cytotoxicity (ADCC) assays were performed using JY cells precoated with anti-HLA mAb (W6/32) (15).

Results and Discussion

Culture of thymocytes in IL-2 results in the rapid generation of non-MHC-restricted CTL, whereas before culture thymocytes do not mediate cytotoxicity (16–19). We have compared IL-2-dependent CD3- γ/δ and CD3- α/β thymic cell lines established from three different individuals for their ability to mediate non-MHC-restricted cytotoxicity and ADCC. Neither the CD3- γ/δ nor CD3- α/β thymic cell lines mediated ADCC activity (Fig. 1), consistent with the absence of Fc receptors (CD16) on these cells (not shown). However, both the CD3- γ/δ and CD3- α/β thymic cell lines lysed an erythroleukemia cell line (K562), a B lymphoblastoid cell line (JY), a colon carcinoma cell line (COLO-205), and a fresh, noncultured sarcoma. Both IL-2-cultured TCR- α/β - and TCR- γ/δ -bearing thymocytes established from the same individuals and grown under identical culture conditions acquired the ability to lyse hematopoietic and solid tumor cell targets without MHC restriction. Moreover, note that both the magnitude and spectrum of target lysis mediated by both the TCR- α/β and TCR- γ/δ thymic cell lines are essentially identical, even though there was no deliberate immunization or selection for cytotoxic activity against these targets. It seems unlikely that an essentially identical repertoire of cytolytic activity would be observed if the TCR- α/β or TCR- γ/δ antigen receptors are responsible for specific antigen recognition. This observation suggests the possibility that a cell surface receptor, other than the TCR, may be responsible for target recognition and cytolysis.

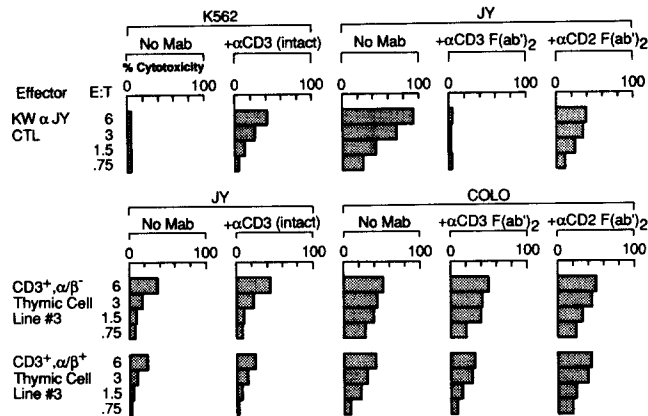
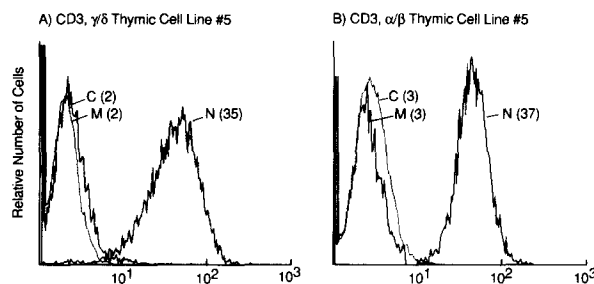


FIGURE 2. Inhibition of cytotoxicity by mAb. CD3- α/β and CD3- γ/δ thymic cell lines and KW anti-JY CTL were assayed for cytotoxicity in the presence or absence of the indicated mAb (5 $\mu\text{g}/\text{ml}$). Preliminary experiments demonstrated that F(ab')₂ fragments of anti-Leu-4 inhibited cytolytic activity of KW anti-JY CTL by >75% at a concentration of 0.5 $\mu\text{g}/\text{ml}$, demonstrating that optimal amounts of blocking antibody were used. Specific cytotoxicity mediated by KW anti-JY CTL against JY was inhibited >90% and 40% by F(ab')₂ fragments of anti-Leu-4 and anti-Leu-5, respectively; whereas, cytotoxicity mediated by the thymic cell lines was not inhibited (<5%).

It has been observed that anti-CD3 antibodies can induce CD3- γ/δ cell lines (5–8), as well as alloantigen-specific CTL cell lines (20), to nonspecifically lyse targets expressing Fc receptors. We have previously demonstrated that intact anti-Leu-4 mAb can induce CD3- γ/δ T cell lines established from normal peripheral blood to nonspecifically lyse JY, a B lymphoblastoid cell line expressing Fc receptors for IgG1 (21). However, intact anti-Leu-4 did not induce nonspecific lysis by either the CD3- γ/δ or CD3- α/β thymic cell lines (Fig. 2). As a positive control for anti-CD3-induced lysis, we demonstrate that anti-Leu-4 induced an alloantigen-specific CTL cell line to nonspecifically lyse K562. These findings indicate that in contrast to CD3- γ/δ PBL/T cell lines and conventional antigen-specific CD3- α/β CTL cell lines, these thymic cell lines did not mediate anti-CD3-induced cytotoxicity. Thus, the CD3/TCR complex on the plasma membrane of these thymic cell lines may not be capable of activating the cytolytic mechanism.

Further studies were undertaken to determine whether cytotoxicity could be inhibited by antibodies against CD3 and CD2. F(ab')₂ fragments of anti-Leu-4 (CD3) or anti-Leu-5b (CD2) did not inhibit the cytotoxicity mediated by either the CD3- γ/δ or CD3- α/β thymic cell lines (Fig. 2). Cytotoxicity was inhibited by anti-CD18 (LFA-1 β) mAb (not shown). However, consistent with prior reports, anti-CD3 and anti-CD2 did substantially inhibit the cytotoxicity mediated by an alloantigen-specific CTL cell line.

Overnight culture of CD3- γ/δ and CD3- α/β thymic cell lines in the presence of F(ab')₂ fragments of anti-Leu-4 (CD3) resulted in substantial modulation of CD3. Quantitation by flow cytometry indicated that >99% of the CD3 antigen was removed from the cell surface of the CD3- γ/δ and CD3- α/β thymic cell lines (Fig. 3). However, modulation of CD3 failed to inhibit cytotoxicity mediated by either the CD3- γ/δ or CD3- α/β thymic cell lines, even though saturating concentrations of anti-CD3 were also present throughout the cytotoxicity assay (Fig. 4). The inability to inhibit cytotoxic function of the CD3- γ/δ and CD3- α/β thymic



relative amounts of fluorescence are indicated in the parentheses as arbitrary linear fluorescence units. Overnight culture of CD3- α/β thymic cells in the presence of F(ab')₂ fragments of anti-Leu-4 completely comodulated WT31 antigen (not shown).

FIGURE 3. Modulation of CD3. CD3- γ/δ thymic cells (A) and CD3- α/β thymic cells (B) were cultured overnight in the absence or presence of F(ab')₂ fragments of anti-Leu-4. The fluorescence histogram of unmodulated cells stained with Ig control mAb (C) is superimposed over the fluorescence histograms of unmodulated cells (N, not modulated) stained with anti-CD3 and modulated (M) cells stained with anti-CD3. Rel-

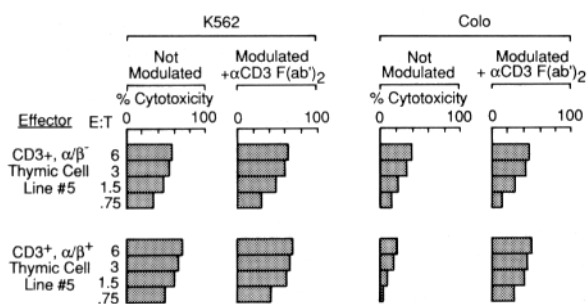


FIGURE 4. Cytotoxicity after modulation of CD3. CD3- γ/δ and CD3- α/β thymic cells were cultured overnight in the absence (not modulated) or presence (modulated) of anti-Leu 4 (Fig. 3). Modulated thymic effector cells were incubated at 37°C for 3 h with F(ab')₂ fragments of anti-Leu-4 (5 μ g/ml) before the addition of targets.

cell lines by anti-CD3 mAb or by modulation of CD3 further questions whether the TCR is responsible for target recognition and signal transduction.

Cytotoxicity mediated by some CD3- γ/δ T cell lines established from peripheral blood is partially inhibited by anti-CD3 antibodies (7–9). These discrepant findings may reflect the origin of these cell lines, i.e., thymus versus peripheral blood. Alternatively, the assay conditions used for blocking may differ. In prior studies, ascites rather than purified mAb was used for inhibition (7–9). It should be noted that data from antibody inhibition experiments must be cautiously interpreted, particularly when using high antibody concentrations. Antibodies against CD2, CD4, CD8, CD11a (LFA-1 α), CD18 (LFA-1 β), and CD45 (T200) can inhibit cytotoxicity mediated by conventional antigen-specific, MHC-restricted CTL; however, these structures are not the antigen receptor.

Non-MHC-restricted cytotoxicity can be mediated by both NK cells and certain T lymphocytes (22). NK cells do not express CD3, and rearrange neither TCR- β nor TCR- γ genes (22–24). In contrast, IL-2-dependent non-MHC-restricted CTL can express either α/β or γ/δ antigen receptors (7–9, 22). Thus, the phenomenon of non-MHC-restricted recognition and cytotoxicity is not exclusively mediated by a single type of effector. NK cells, as well as CD3- γ/δ - and CD3- α/β -bearing T cell lines, recognize and kill a similar spectrum of tumor cell targets. Results from our present studies indicate that some CD3- α/β and CD3- γ/δ thymocytes may not mediate cytotoxicity via the CD3/TCR complex. These observations suggest that NK cells and some non-MHC-restricted CTL may mediate cytotoxicity by a common target recognition structure other than the TCR.

In conclusion, the only evidence that CD3- γ/δ T cells recognize target and

mediate non-MHC-restricted cytotoxicity via the TCR- γ/δ is based on blocking with anti-CD3. Since we have demonstrated that cytotoxicity mediated by both CD3- γ/δ and CD3- α/β thymic cell lines is not affected by modulation of the CD3 complex and is not inhibited by saturating concentrations of purified, F(ab')₂ anti-CD3, these results indicate that the CD3/TCR complex may not be required for cytotoxicity. Therefore, the possibility that cytotoxicity is mediated by a receptor other than the TCR must be considered until there is formal proof by genetic reconstitution that the TCR- γ/δ or - α/β is necessary for non-MHC-restricted cytotoxicity.

Summary

After culture in IL-2, thymocytes expressing either TCR- α/β or - γ/δ acquired the ability to lyse hematopoietic and solid tumor cell targets without deliberate immunization or apparent restriction by the MHC. Moreover, TCR- α/β - and TCR- γ/δ -bearing thymic cell lines demonstrated an essentially identical spectrum of cytolysis against several tumor cell targets. Cytotoxicity was not inhibited by antibodies against CD3 or CD2 and modulation of the CD3/TCR complex also failed to affect cytotoxicity. Thus, non-MHC-restricted cytotoxicity can be mediated by thymocytes with either TCR- α/β or TCR- γ/δ , but the TCR may not be responsible for target recognition.

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